# NATURE AND OCCURRENCE OF JUGLONE IN *JUGLANS NIGRA* L.

by

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TABLE OF CONTENTS

| | Page |
|---|---|
| INTRODUCTION | 1 |
| REVIEW OF LITERATURE | 2 |
| MATERIALS AND METHODS | 7 |
| Identification of Juglone | 7 |
| Extraction and Purification | 7 |
| Chromatography | 7 |
| Microscopic Examination | 8 |
| Infrared Spectrophotometry | 9 |
| Quantitative Estimation of Juglone | 9 |
| Extraction | 9 |
| Spectrophotometric Measurements | 9 |
| Comparison With the Standard Curve | 9 |
| Dry Matter of the Leaves, Hulls, and Roots | 10 |
| RESULTS | 10 |
| DISCUSSION | 23 |
| SUMMARY | 29 |
| ACKNOWLEDGMENTS | 31 |
| REFERENCES | 32 |

# INTRODUCTION

The problem of walnut toxicity was first mentioned by Pliny in his "Natural History." He believed the shadow of the walnut to be poisonous to all plants and killed whatever it touched.

This old problem was investigated by many scientists in the early twentieth century. The results, however, were divided: some cited evidences of the incompatibility of various crops with walnut trees (Jones and Morse, 1902; Cook, 1921; Massey, 1925; Pirone, 1938; Reinking, 1943), while others failed to find concrete evidence of such incompatibility (MacDaniels and Muenscher, 1940; Mattoon, 1942).

Considerable effort has been spent in an effort to find in which part of the walnut tree the toxic material is produced. Cook (1921) and Massey (1925) observed from field studies as well as water cultures that the toxic material was from the root of the walnut trees. Hans Bode (1958), German botanist, believed that the toxicity came from the leaves of the walnut. However, he did not give an explanation of why roots failed to exhibit the toxic effect in pot trials under glass while they apparently produced an inhibitory effect in water culture. Further investigation is needed in order to find a satisfactory answer to this problem. The existence of juglone in the walnut is firmly established though the relative amount of juglone in various species of Juglans has not been completely investigated (Gries, 1943).

The objective of this experiment was to develop a technique for the comparative study of the juglone content in different parts of the tree and in selected cultivars of Juglans nigra as well as to determine the monthly variation in juglone content.

## REVIEW OF LITERATURE

The black walnut occurs naturally throughout a wide area of eastern North America. It is found from northern New England through southern Canada and west to South Dakota; then southward to central Texas; eastward to central Georgia; then northward, reaching the Atlantic coast in South Carolina. This species does not occur at high elevation in the Appalachian mountain region, but elsewhere it is well distributed throughout its botanical range. It grows best in the moist, rich soils that are characteristic of such areas. Black walnut is a medium to large tree. Seedlings develop a deep tap-root, as the tree grows; a heavy lateral root system also is produced. The lateral roots are usually well under-ground (Brook, 1951).

The toxic effects of walnut was noticed early in human history. Much of the work on walnut toxicity was done in the early twentieth century. Schneiderhan (1927) reported that apple trees have been observed to be stunted and to eventually die when their roots come in contact with those of walnuts. Perry (1932) investigated and made careful measurements on the number of white pine which died or were stunted while growing in association with black walnuts. Smith (1942) pointed out the scarcity or absence of broomsedge and poverty grass under walnut trees. Pirone (1938) investigated injuries done by black walnuts to rhododendron and other Ericaceous plants. Jones and Morse (1902) found apparent antagonism between walnut and shrubby cinquefoil. Reinking (1943) reported that cabbage and tomato plants were wilted and stunted when planted close to black walnut trees. On the other hand,

MacDaniels and Muenscher (1940) cited evidences, pro-and-con, relative to the toxic effect of black walnut on various crops. They concluded that the toxic substance is unstable and quickly destroyed in the soil; such destruction possibly being related to soil aeration. Mattoon (1942), at the same time, failed to find evidence of allelopathy between soybean, apple, peach and walnut.

Cook (1921) reported on the wilting of tomato plants growing in the vicinity of black walnut trees from outdoor observations. He found that the occurrence of the wilting of tomato plants coincided with the distribution of the root system of the tree. Massey (1925) placed root bark of black walnuts in a water culture of tomato plants and found that the plants wilted within 48 hours. He concluded that the root bark contained a toxic substance and suggested that the toxic constituent in the walnut tree might be juglone.

A similar experiment was performed by Brown in 1942. In a water culture of tomato plants with root bark of walnut added, he found the dry weight of the plants was decreased. The injurious effect was also shown by germinating seeds in contact with walnut root bark.

Previously, the common belief of a toxic material coming from the roots of the tree was widely accepted. In 1958, Hans Bode showed that an inhibitory effect on the growth of tomato plants came from an excretion from the leaves carried down by rain rather than from the roots of the walnut trees. He found from a pot trial, that when the tomato plants were protected from the run-off of rain from the leaves of walnut tree, the curling of the leaves and the yellowing of the lower leaves of the tomatoes usually seen did not occur. Also nutrient solutions previously

used for the growing of walnut seedlings promoted the growth of tomatoes. He concluded that rain-water drip from leaves and fallen leaves contained an inhibitory substance (juglone) of lasting effect.

Davis (1928) extracted the toxic substance from Juglans nigra and found that the purified plant extract was chemically identical with synthesized juglone. Similar toxicity has been exhibited as a result of both the synthetic and natural products being injected into tomato plants. He made a study of the oxidation and reduction system of juglone in 1931, and suggested that the resistance to walnut toxicity might be associated with the reduction of toxic juglone to non-toxic hydrojuglone.

Juglone was found in different parts of the tree in the Juglandaceae family. It was reported toxic to the lower forms of plant life such as molds, fungi and bacteria. The high concentration in the reproductive organ and buds led Daglish (1950 b) to believe that juglone might act as a protective agent to ensure the success of the reproductive cycle and the appearance of the shoots for the following years.

The occurrence of juglone in the walnut was first reported in 1856 by Vogel and Reischauer, who gave the name nucin to the yellow crystalline compound isolated from the pericarp. Bernthsen and Semper (1887) determined its structure as 5-hydroxy-1,4-naphthoquinone which was confirmed by its synthesis. Mylius (1885) had reported that juglone existed as alpha-hydrojuglone and beta modification in the walnut. Daglish (1950 a) reported that juglone existed in the walnut as the glucoside of 1,4,5-trihydroxynaphthalene. On hydrolysis, it yields glucose and alpha-hydrojuglone. Gries (1943) stated that the non-toxic reduced form of juglone in the walnut can be changed to the toxic form upon

exposure to the air. Upon standing in the air juglone again disappeared, being either changed back to hydrojuglone or broken down into other non-toxic substances.

Juglone has a molecular weight of 174.15, and occurs as yellow needles from benzene and petroleum ether mixture. It melts at 155° C and sublimes readily. It is slightly soluble in hot water, soluble in alcohol and ether, freely soluble in chloroform and benzene (Auyong, 1962). It has a characteristic irritative odor, and possesses the following structure:

The absorption spectra of juglone in methanol obtained by Spruit (1955) showed that there were absorption peaks at 250 mu and at 420 mu. The spectra were measured by a photoelectric method. The absorption spectra obtained by Horitsu (1956) in chloroform with a Beckmann DU spectrophotometer showed that the absorption peak was at 430 mu.

Horitsu (1956) used ether to extract juglone from Juglans regia and Juglans sieboldiana Maxim. Distilled water, ether, acetone, unleaded gasoline, heptane and petroleum ether were tried as extracting solvents for juglone. Ether and petroleum ether were found to be the best solvents to extract juglone from fresh hulls (Auyong, 1962).

Sublimation was used as a method of purification of juglone (Horitsu, 1956; Auyong, 1962). The dried crude plant extract was placed in an evaporating dish, which was covered by a watch glass on a sand bath. The

temperature was kept at about 100° C. The juglone was sublimed to the watch glass in a relatively pure form (Auyong, 1962).

The literature for the chromatography of juglone is quite limited. Sproston (1954) used a paper chromatographic solvent composed of amyl alcohol, pyridine and water in the ratio of 3 to 2 to 1.5 to separate some substituted naphthoquinones. Pannell and Luvalle (1953) used a solvent composed of butanol, acetic acid and water mixed in the volume proportion of 4 to 1 to 5 from which the organic layer is used to test hydroquinone and some of its derivatives for photographic developer. N-butyl alcohol and water (150:25) was used by Kuroda as a chromatographic solvent in 1955. This was found a suitable solvent for the one-dimensional ascending method. Toyo-Filter paper No. 50 previously treated with 6N HCl aqueous solution was used in the process. Chromatography was carried out in glass cylinders at 25° C for about nine hours. The Rf value of juglone thus obtained was 0.91.

Sproston (1954) used five percent aqueous solution of NaOH to detect substituted naphthoquinones. Horitsu (1956) reported that juglone produced a red color in $NH_4OH$ solution. Juglone in diluted HCl and diluted NaOH solution was found to be light yellow in the former and red in the latter solution. The color reactions of juglone reported by Kuroda (1955) were brownish purple under ultra-violet rays, reddish brown with magnesium bicarbonate, light brown with sodium bicarbonate, and reddish purple with caustic soda.

# MATERIALS AND METHODS

## Identification of Juglone

Two cultivars of black walnut (Juglans nigra L.), Thomas, Ohio, and seedlings grown at the Horticultural farm, Manhattan, Kansas, were chosen for the experiment. Four samples of hulls and leaves were taken randomly from each cultivar and seedling, two from the upper part and two from the lower part of the trees. After the samples were taken, they were kept in a freezer until extractions were made.

Samples were collected at approximately monthly intervals on June 30, July 26, August 26, and September 24, 1966. Root samples of two-year old seedlings were collected on September 24 the same year.

Extraction and Purification. Six grams of a frozen sample were weighed and put in a glass thimble with 50 ml. of petroleum ether. The solvent was measured into a pyrex beaker which was tightly sealed around the thimble in a Goldfisch solvent extractor manufactured by Laboratory Construction Company, Kansas City, Missouri. The sample was extracted for six hours.

The pyrex beaker was removed from the extraction apparatus after six hours and the petroleum ether evaporated on a hot plate. The dried crude extract was transferred to an evaporating dish which was covered by a watch glass. The evaporating dish was placed on a "Thermolyne" hot plate, and heated at about 100° C until a yellowish brown compound was sublimed to the surface of the watch glass.

Chromatography. The compound collected was dissolved in 5 ml. of chloroform. The ascending chromatographic method as described by Kuroda

and Harada (1955) was used. About 20 lambda of the solution was spotted on the starting line of the strips of Whatman No. 1 filter paper previously treated with 6N HCl aqueous solution. To prevent the spreading of the spots, a stream of gentle air was blown over the paper strips to make the spots as small as possible.

The spotted strips were next placed in glass cylinders containing n-butyl alcohol and water in the ratio of 150 to 25 as a chromatographic solvent, and chromatographed at 25° C for about six hours. The papers then were dried at room temperature, the dried chromatograms examined with ultra-violet light, sprayed, and the distance from the center of the original spots and which the solvent fronts had travelled were measured, and Rf values determined.

The Rf value thus obtained represented the average of three strips of a sample, and was compared with the Rf value of the commercial purified juglone purchased from Mann research Laboratories in New York, N. Y.

The compound was detected by spraying the paper strip with five percent sodium hydroxide solution. The characteristic color change appeared on the chromatograms would show the location and concentration of the compound.

Microscopic Examination. The crystals obtained from the purified plant extract were examined under a microscope. The color and forms of the crystals were carefully compared with that of commercial purified juglone which was resublimed and examined under the same conditions as the plant extract.

Infrared Spectrophotometry. In the further identification of the compound thus obtained, infrared spectrophotometry was utilized using a Perkin-Elmer 137 sodium chloride spectrophotometer. Eight grams of frozen sample, each of leaves, hulls, and roots were used for the extraction. The extracts then were evaporated, purified and dissolved in 2 ml. of chloroform. Infrared spectra were taken on salt plates using one ml. of each preparation.

## Quantitative Estimation of Juglone

Quantitative estimation of juglone was carried out in the following four steps:

Extraction. Two gram frozen samples were extracted with 50 ml. of petroleum ether for six hours in the solvent extractor, and a clear yellowish solution was obtained.

Spectrophotometric Measurements. The absorption spectra of juglone were determined with a Beckman DB automatic spectrophotometer from 380 to 480 millimicrons, and the point of maximum absorption was determined. The quantity of juglone in plant extract prepared in the above manner was determined at the wavelength of 410 mu with a Beckman DU spectrophotometer. The plant extracts were diluted to the point that all readings would fall in the sensitive area of the spectrophotometer. Three readings were made for each extract. The average of the three was taken as the optical density of the extract in question.

Comparison With the Standard Curve. A ten-milligram sample of commercial purified juglone was placed in a volumetric flask of 50 ml. capacity and enough petroleum ether added to make up to volume.

A complete dissolution was necessary before samples of 2, 4, and 6 ml. of solution were drawn and transferred to three different 50 ml. volumetric flasks. They were diluted to volume with petroleum ether and readings of optical density were taken at the wavelength of 410 mu of a Beckman DU spectrophotometer. Three points thus obtained were plotted on a graph paper. Those points showed the linear relationship with concentration as shown in Plate I. The quantity of juglone of the unknown solution could be estimated by comparison with the standard curve.

Dry Matter of the Leaves, Hulls, and Roots. To determine the dry matter, random samples of leaves and hulls from a specific area of the tree were taken. Four gram samples were weighed and placed in an oven at 75° C for 24 hours. Ten minutes after the samples were removed from the oven, they were weighed again and the percentages of the dry matter of the samples were calculated.

## RESULTS

### Identification of Juglone

Chromatography. The paper strip ascending method of chromatography, using the solvent system of n-butyl alcohol and water, was carried out to identify the juglone. The commercial purified juglone was chromatographed under the same condition as the controls, and was found to have an average Rf value of .89. The average Rf values of the plant extracts are shown in Table 1.

## EXPLANATION OF PLATE I

X-axis represents milligrams of juglone per 50 milliliters in petroleum ether.

Y-axis represents absorbance with a Beckman DU spectrophotometer.

The quantity of juglone in an unknown sample could be obtained by converting the optical density to milligrams of juglone in 50 milliliters of the extract in petroleum ether.

PLATE I

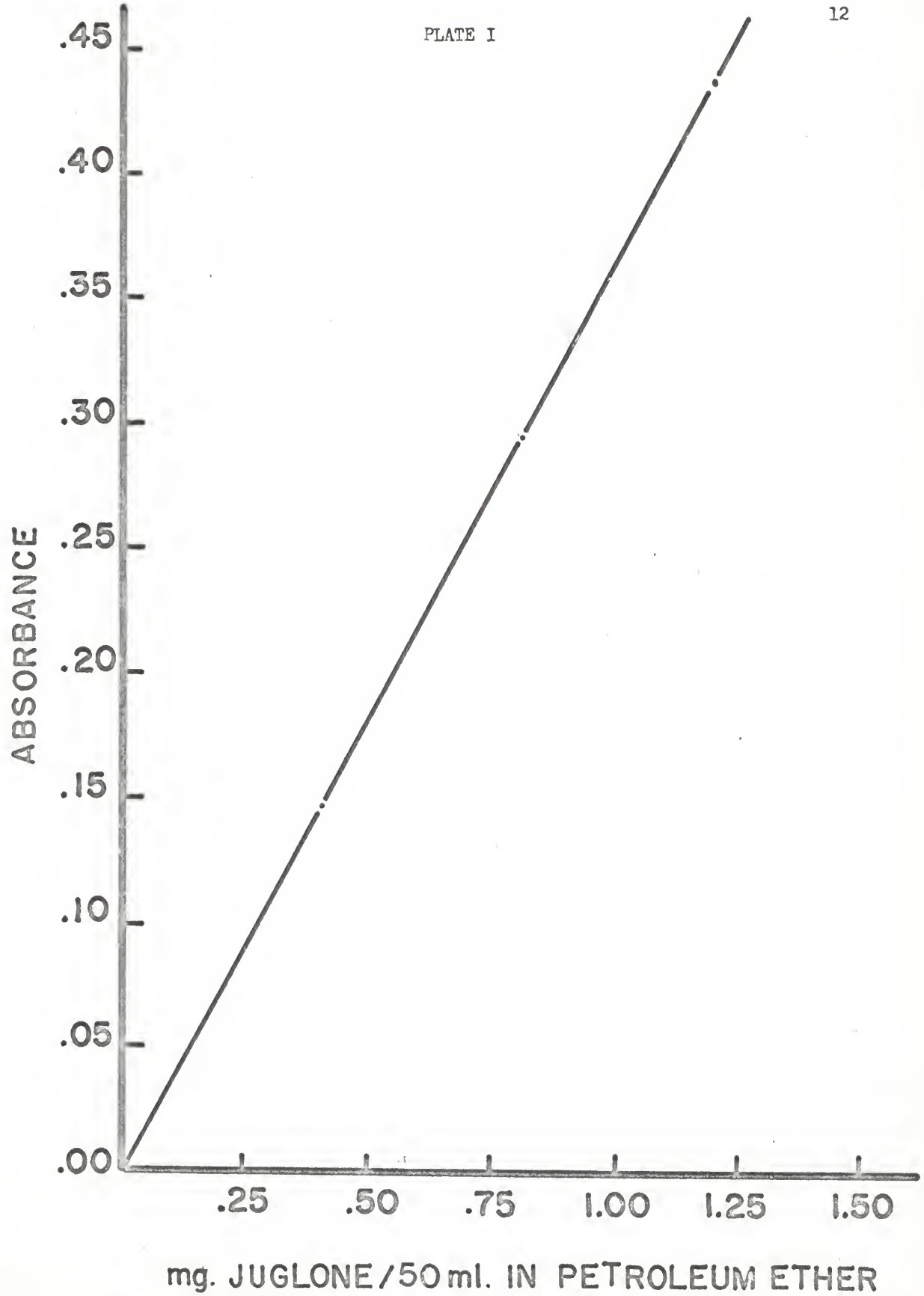
.45
.40
.35
.30
.25
.20
.15
.10
.05
.00
ABSORBANCE
.25
.50
.75
1.00
1.25
1.50
mg. JUGLONE/50 ml. IN PETROLEUM ETHER

Table 1. Rf values of juglone in n-butyl alcohol and water extracts obtained from leaves, hulls, and roots.

| Organ | Cultivar | | |
|---|---|---|---|
| | Thomas | Ohio | Seedlings |
| Leaves | .89 | .89 | .88 |
| Hulls | .88 | .88 | .88 |
| Roots | -- | -- | .89 |

The spot which appeared on the dried chromatogram was yellow in color. It was brown-purple under the ultra-violet light. A reddish-purple color appeared when sprayed with five percent sodium hydroxide solution, and light brown color when sprayed with five percent sodium bicarbonate solution.

Microscopic Examination. The crystals of the purified compound from the plant extracts were found to be long and pointed, and could be termed needle shaped. Under certain conditions, it had the form of a feather. All of them were orange brown in color and similar in form to the crystals of the commercial purified juglone.

Infrared Spectrophotometry. The purified plant extracts of leaves, hulls, roots, and commercial purified juglone were examined with an infrared spectrophotometer. The spectra obtained appeared to be from identical compounds with similar absorption peaks. The infrared spectrum of the commercial purified juglone is shown in Plate II.

EXPLANATION OF PLATE II

Infrared absorption curves for the

commercial purified juglone in chloroform.

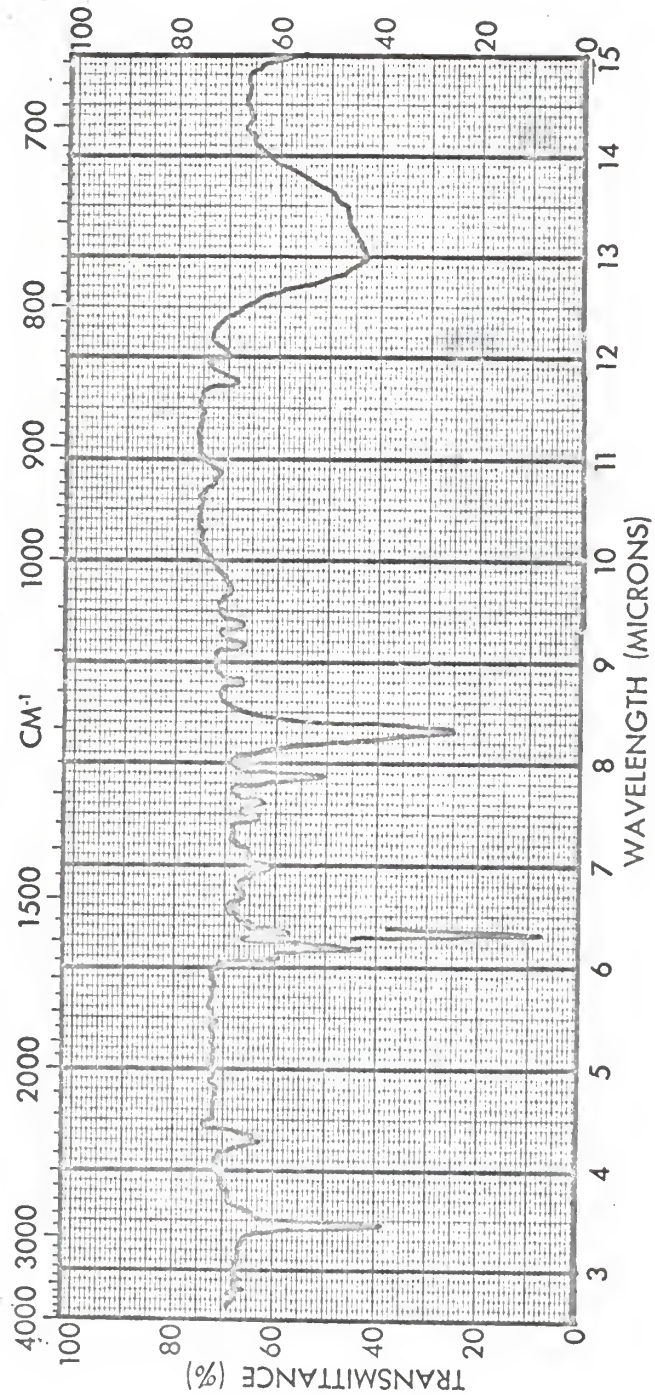
TRANSMITTANCE (%)
100
80
60
40
20
0
4000
3000
2000
1500
1000
900
800
700
CM-1
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH (MICRONS)

PLATE II

## Quantitative Estimation of Juglone

The absorption spectra of plant extracts were found to be similar with that of the commercial purified juglone over the range of 380 to 480 mu (Plates III, IV, and V). They were found to have maximum absorption values at 410 and 420 mu. In order to decide the degree of interference that the impurities in the extract might have upon the absorption values, six grams of plant parts were extracted and purified by sublimation. The juglone as well as the residue obtained from the above sample were dissolved in petroleum ether and read at 410 and 420 mu. The interference of impurities from the residue was found to be less than 1.2 percent.

The juglone content of each cultivar and the seedling was compared using one gram dry samples. It was found that in the case of hulls, Ohio had the highest juglone content, and seedlings had the least, while Thomas hulls had a content somewhere in between. In the case of leaves, Thomas had the highest juglone content, Ohio had the least, and the seedlings had a content somewhere between these two cultivars.

It was also found that there were differences in juglone content between the leaves and hulls of each cultivar and seedling. The hulls generally had higher concentrations than leaves. The difference in juglone content among the three parts of the seedlings were found to be significant in the month of September. The roots in that month had the greatest amount of juglone with hulls next in concentration, and leaves with the least.

## EXPLANATION OF PLATE III

The absorption spectra of hull extract and the commercial purified juglone in petroleum ether referred to here as the standard. The maximum absorption values in both cases occur from 410 to 420 millimicrons.

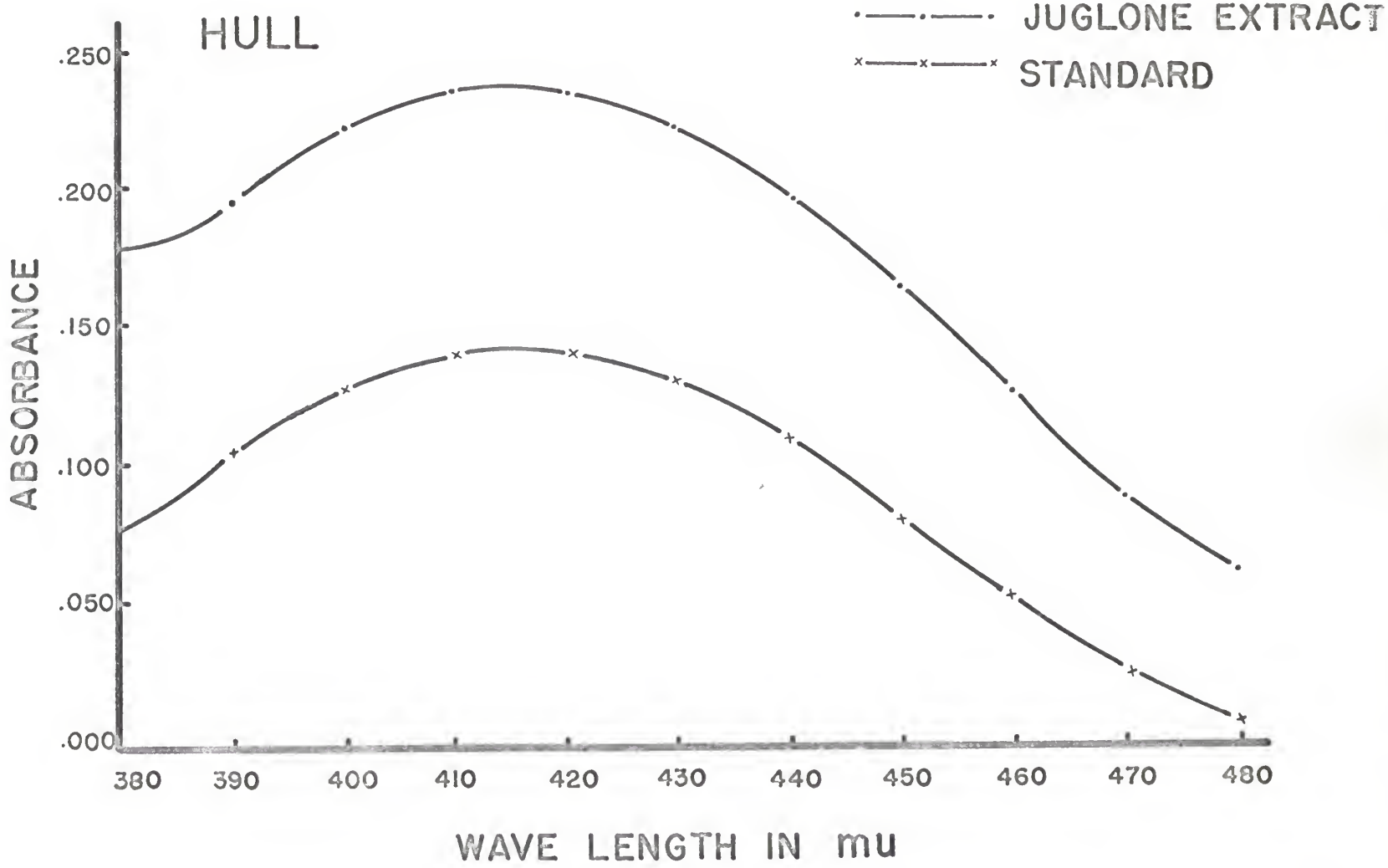
HULL
JUGLONE EXTRACT
STANDARD
ABSORBANCE
.250
.200
.150
.100
.050
.000
380
390
400
410
420
430
440
450
460
470
480
WAVE LENGTH IN mu

PLATE III

## EXPLANATION OF PLATE IV

The absorption spectra of leaf extracts and the commercial purified juglone in petroleum ether. The maximum absorption values are from 410 to 420 millimicrons.

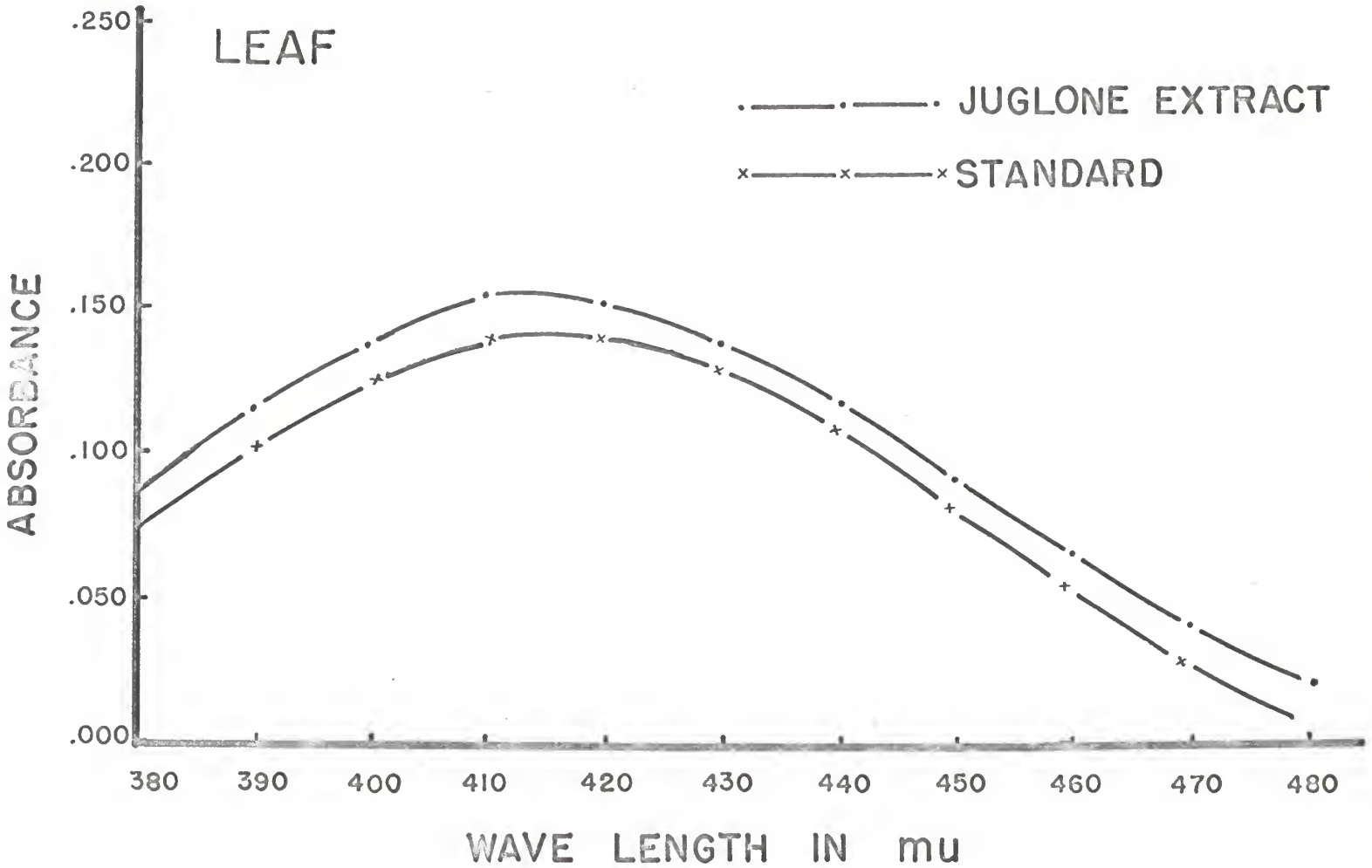
LEAF
JUGLONE EXTRACT
STANDARD
ABSORBANCE
.250
.200
.150
.100
.050
.000
380
390
400
410
420
430
440
450
460
470
480
WAVE LENGTH IN mu

PLATE IV

## EXPLANATION OF PLATE V

The absorption spectra of root extracts and the commercial purified juglone in petroleum ether. The maximum absorption values are from 410 to 420 millimicrons.

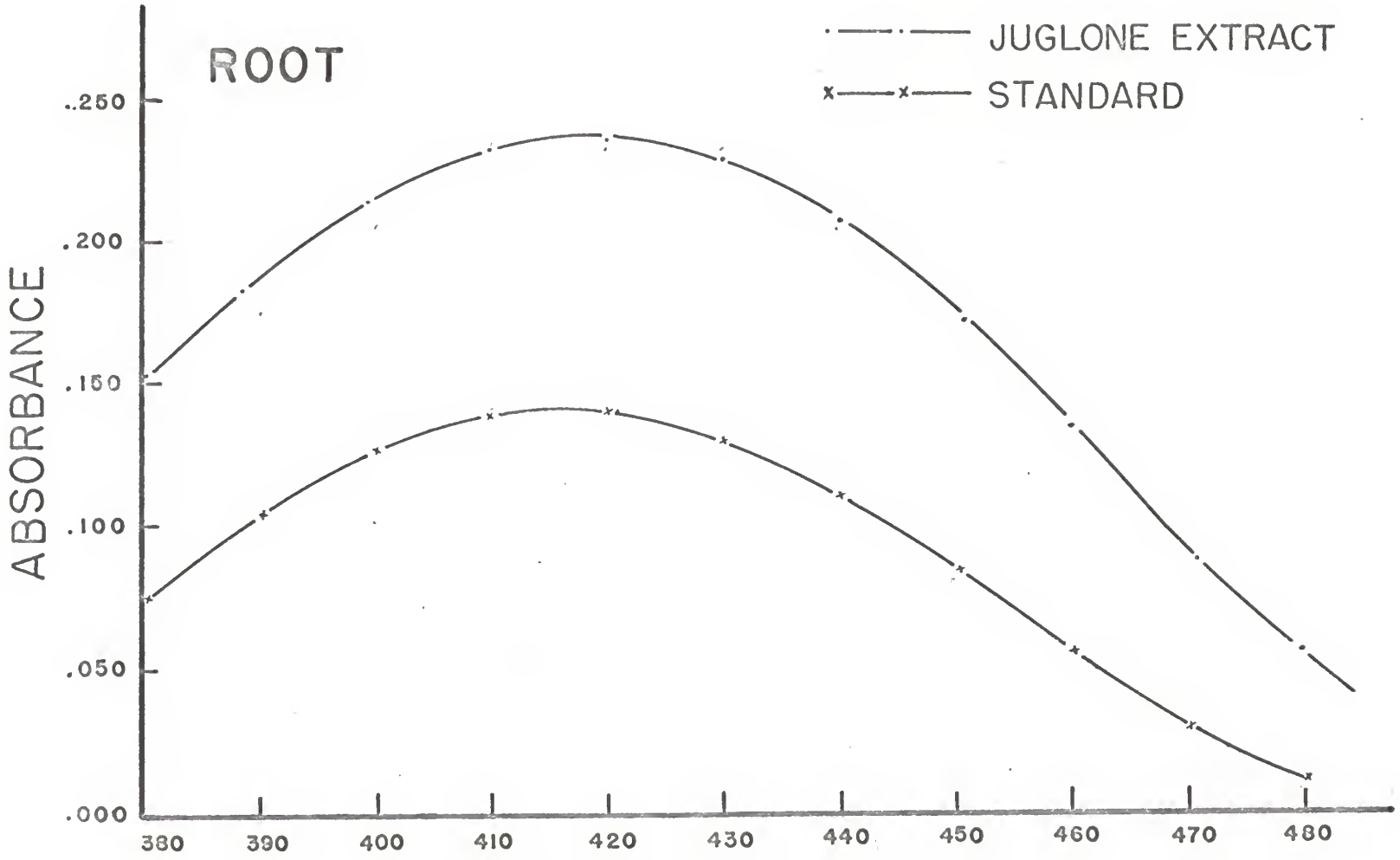
ROOT
JUGLONE EXTRACT
STANDARD
ABSORBANCE
.250
.200
.150
.100
.050
.000
380
390
400
410
420
430
440
450
460
470
480
WAVE LENGTH IN mu.

PLATE V

No difference was found between samples collected from the upper and lower positions on the trees, and no interaction was found between positions and cultivars. The change of juglone content during the 4 month period was found to be significant in Ohio and seedlings. The leaves of seedlings had highest juglone content in July, and lowest juglone content in September. The leaves of Ohio showed the same variation between months. The hulls of seedlings had the highest juglone content in August, and the lowest in June. The hulls of Ohio had the highest juglone content in September, and lowest in June. The variation was not significant in either leaves or hulls in the Thomas cultivar. The results of these comparisons are shown in Tables 2, 3, 4, 5, 6 and 7.

## DISCUSSION

Kowkabany et al. (1952) investigated various factors that may affect Rf value in paper chromatography and found that the Rf value decreased as the initial spot is placed at an increasing distance from the surface of the developer. They also found that the concentration of solution applied to be negatively correlated with Rf value. These findings may be suitable to explain the difference of the Rf value obtained in this experiment (Table 1). The average Rf value of juglone obtained by Kuroda (1955) was 0.91; a difference of 0.02 from the results of this experiment. This may have been due to the different filter paper used. The time required for the development of chromatography was different between the two treatments. The 6N HCl treated paper required about 6 hours but the untreated paper needed more than 8 hours.

Table 2. Average value of juglone in milligrams per gram of dry hulls.

| Month | Cultivar: Ohio | Thomas | Seedling | LSD 5% | F |
|---|---|---|---|---|---|
| June | 9.6474 | 9.2825 | 4.4095 | 1.4053 | 52.04** |
| July | 13.9653 | 10.2594 | 4.4778 | 2.2060 | 56.40** |
| August | 14.8951 | 11.5405 | 9.4197 | 3.4124 | 7.86* |
| September | 16.5425 | 10.9541 | 6.7080 | 4.3600 | 15.36** |
| LSD 5% | 2.8432 | ----- | 1.2808 | | |
| F | 10.18** | 1.34 ns | 33.41** | | |

A two-way analysis of variance of above data is presented below:

| | June | | July | |
|---|---|---|---|---|
| Source | df | Mean square | df | Mean square |
| Cultivars | 2 | 34.2449** | 2 | 91.4489** |
| Positions | 1 | 0.6595 | 1 | 1.1773 |
| C × P | 2 | 0.1356 | 2 | 2.5658 |
| Sample | 6 | 0.6580 | 6 | 1.6213 |
| | August | | September | |
| Source | df | Mean square | df | Mean square |
| Cultivars | 2 | 30.4880* | 2 | 97.3279** |
| Positions | 1 | 1.0373 | 1 | 4.3377 |
| C × P | 2 | 1.0696 | 2 | 1.1534 |
| Sample | 6 | 3.8795 | 6 | 6.3348 |

* Indicates 5% level of significance.
** Indicates 1% level of significance.
ns Indicates nonsignificant results.

Table 3. Average value of juglone in milligrams per gram of dry leaves.

| Month | Cultivar: Thomas | Seedling | Ohio | LSD 5% | F |
|---|---|---|---|---|---|
| June | 2.8984 | 1.4930 | 1.2081 | 1.1025 | 7.58* |
| July | 2.7508 | 2.2762 | 1.5386 | 0.6904 | 9.41* |
| August | 2.4615 | 1.2825 | 1.2228 | 0.9126 | 10.34* |
| September | 1.7614 | 1.2245 | 0.5534 | ---- | 4.30 ns |
| LSD 5% | ---- | 0.4295 | 0.3011 | | |
| F | 1.29 ns | 12.14** | 17.48** | | |

A two-way analysis of variance of above data is presented below:

| | June | | July | |
|---|---|---|---|---|
| Source | df | Mean square | df | Mean square |
| Cultivars | 2 | 3.2758* | 2 | 1.4926* |
| Positions | 1 | 0.4891 | 1 | 0.3816 |
| C × P | 2 | 0.3000 | 2 | 0.0574 |
| Sample | 6 | 0.4323 | 6 | 0.1587 |
| | August | | September | |
| Source | df | Mean square | df | Mean square |
| Cultivars | 2 | 1.9518* | 2 | 1.4654 ns |
| Positions | 1 | 0.7333 | 1 | 0.5428 |
| C × P | 2 | 0.4008 | 2 | 0.2659 |
| Sample | 6 | 0.1888 | 6 | 0.3410 |

* Indicates 5% level of significance.
ns Indicates nonsignificant results.

Table 4. The relative concentration of juglone in the leaves and hulls of the Thomas cultivar.

| Organ | Month | | | |
|---|---|---|---|---|
| | June | July | August | September |
| Hulls | 9.2825 a | 10.2594 a | 11.5405 a | 10.9541 a |
| Leaves | 2.8984 b | 2.7508 b | 2.4615 b | 1.7614 b |

Values (within a given column) designated by the different lower case letters are significantly different at the 5% level as determined by T Table.

Table 5. The relative concentration of juglone in the leaves and hulls of the Ohio cultivar.

| Organ | Month | | | |
|---|---|---|---|---|
| | June | July | August | September |
| Hulls | 9.6474 a | 13.9653 a | 14.8951 a | 16.5425 a |
| Leaves | 1.2081 b | 1.5386 b | 1.2228 b | 0.5534 b |

Values (within a given column) designated by the different lower case letters are significantly different at the 5% level as determined by T Table.

Table 6. The relative concentration of juglone in the leaves and hulls of the seedling samples.

| Organ | Month | | | |
|---|---|---|---|---|
| | June | July | August | September |
| Hulls | 4.4069 a | 4.4778 a | 9.4197 a | 6.7080 a |
| Leaves | 1.4930 b | 2.2762 b | 1.2825 b | 1.2245 b |

Values (within a given column) designated by the different lower case letters are significantly different at the 5% level as determined by T Table.

Table 7. The relative concentration of juglone in the leaves, hulls, and roots of the seedlings for the month of September.

| Organ | Samples | | | | LSD 5% | F |
|---|---|---|---|---|---|---|
| Leaves | 1.2580 | 1.0333 | 1.2580 | 1.3488 | | |
| Hulls | 6.8390 | 7.4073 | 6.2573 | 6.3285 | 0.8428 | 172.88** |
| Roots | 7.4389 | 8.8102 | 7.4556 | 7.2111 | | |

** Indicates 1% level of significance.

Apparently, the treatment increased the rate of flow of the solvent in this experiment. The n-butyl alcohol and water system produced a clear-cut round spot, but the Rf value was very high. Efforts were made to vary the solvent systems used. This included the adding of butyl acetate, acetic acid, and the adjustment of the water ratio. No significant difference in Rf values were found from using these modifications.

The other solvent tried in this experiment was a mixture of amyl alcohol, pyridine and water. It was difficult to ascertain the Rf values in this solvent system because of tailings which occurred on the chromatograms.

From the results of Rf values, color reactions, microscopic examinations and infrared spectra determinations, it appeared that the plant extracts were similar to commercial purified juglone.

The quantitative estimation was, however, faced with the possibility of interference of impurities, especially from the carotenoids. This was ruled out by the agreement of the absorption spectra from 380 to 480 mu of the plant extracts with that of the commercial purified juglone. Sublimation of plant extracts showed that the interference at 410 and 420 mu was negligible.

The absorption maxima found in this experiment was 410 to 420 mu. This value was slightly different from the absorption spectra of the authentic juglone described in the literature. The difference may have been due to the different solvents used in each case.

The results of the quantitative estimation showed there were differences in juglone content among the different cultivars, however, there was no positive correlation in occurrence of juglone between the

different parts of a cultivar.

The monthly variation of juglone in the hulls partly agree with results of the experiment run by Daglish (1950 b) who attempted to determine the hydrojuglone glucoside in the walnut. The first part of his experiment designed for the flowering stage was not covered in this experiment. He stated that the highest concentration of hydrojuglone glucoside occurred in tissues concerned with the reproductive cycle, after fertilization of flower and the maturation of the fruit are accomplished. No suitable conclusion can be drawn concerning the variation of juglone content of leaves.

## SUMMARY

Juglone has been found in various parts of trees in the Juglandaceae family, and the black walnut has been found to have a high concentration of this toxic organic compound. This experiment was designed to develop a technique for the quantitative estimation of the juglone content in black walnuts and to determine the monthly variation of juglone in selected parts of the plant.

Samples of leaves and hulls were collected at approximately monthly periods from June to September in 1966. Root samples of two-year old seedlings were collected in September the same year.

The frozen samples were extracted with petroleum ether, and their juglone contents were determined by spectrophotometric measurements.

The results showed that in the case of leaves, Thomas had the highest, Ohio had the lowest, and the seedlings a content intermediate between the two cultivars. Ohio had the highest juglone content for

the hulls, Thomas second in quantity and the seedlings had the lowest. The hulls were found to have a higher juglone concentration than the leaves in each case. The juglone concentration in roots was found to be greater than for the hulls and leaves of the seedlings for the month of September. There were significant variations in juglone content during the four month period in the Ohio cultivar and the seedlings. The leaves of seedlings in July contained juglone at the maximum and in September at the minimum level. The leaves of Ohio cultivars showed almost the same trend. The juglone level in hulls of seedlings peaked in August, and was lowest in June, while for the Ohio cultivar the highest values were obtained in September, and the lowest in June. No significant difference in juglone content in either leaves or hulls of Thomas cultivar were observed at any of the 4 monthly sampling dates.

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# NATURE AND OCCURRENCE OF JUGLONE IN *JUGLANS NIGRA* L.

by

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The toxic effect of walnut trees on the tomato, alfalfa and other plants has been reported in many cases. This has been found due to the existance of juglone in the trees.

The relative amounts of juglone in the different cultivars of Juglans nigra has not been completely investigated. This experiment was designed to develop a technique for the quantitative estimation of the juglone content in the cultivars Ohio, Thomas and seedling walnuts, and to determine the monthly variation of juglone in selected portions of walnut trees.

The purified plant extracts were identified by Rf value, color reactions, color and shape of the crystals as well as infrared spectra, and found to be similar to the commercial purified juglone.

The juglone identified was estimated quantitatively by using a standard curve prepared for this purpose. The absorption spectra and peak of absorption were determined before the estimations were made. The juglone content of individual samples were read at the wavelength of 410 millimicrons.

The juglone content of each cultivar and from the seedling sample was compared on the basis of one gram of dry sample. In hulls, Ohio had the highest juglone content, and the seedlings had the lowest, while Thomas hulls had a content somewhere in between. Thomas leaves had the highest juglone content, Ohio the least, and the leaves of the seedlings had a content somewhere between these two cultivars.

It was also found that there were differences in amount of juglone between the leaves and hulls of each cultivar and seedlings. The hulls generally had higher concentration than leaves. The difference in

juglone content among the three parts of the seedlings were found to be significant in the month of September. The roots in that month had greatest amount of juglone with hulls next in concentration, and leaves with the least.

No difference was found in the amount of juglone in leaves from the upper and lower sampling positions on the trees, and no interaction was found between positions and cultivars. The variation in juglone content during the four month period was found to be significant in the Ohio cultivar and seedlings. The leaves of seedlings and the Ohio cultivar had the highest juglone content in July, and the lowest in September. The seedling hulls had the highest juglone level in August, and the lowest in June, while for the Ohio cultivar the highest values were obtained in September, and lowest in June. The variation in juglone content was not significant in either leaves or hulls of Thomas cultivar at any of the 4 monthly sampling dates.